prising:

(a) obtaining a fresh sample of lipid containing biological fluid;

(b) measuring the amount of noncyclooxygenase derived prostanoids in the sample within about two hours of obtaining the sample, which is the amount of time it takes for [prior to] the *ex vivo* development of prostanoids in the sample;

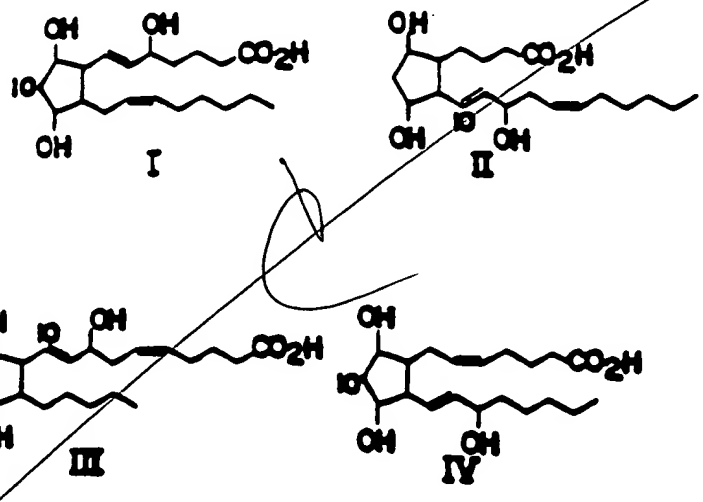
(c) comparing said measured amount of prostanoids with a control; and

(d) assessing oxidative stress *in vivo* based on the comparison in step c.

Please cancel claims 2 and 3.

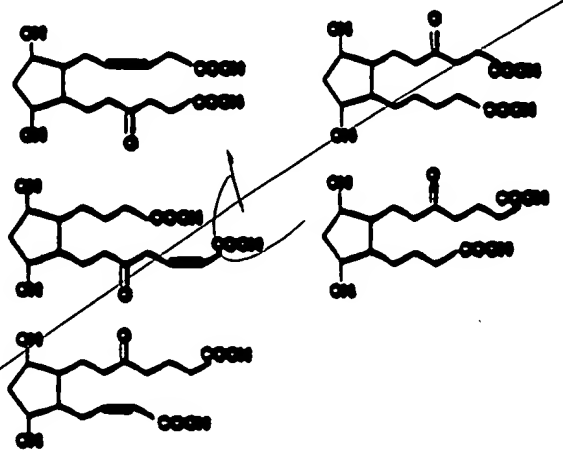
Please amend claim 4 as follows:

~~4. The method of any of claims 1, 6, 7, 10, 11, 14, 15, and 18~~ wherein said prostanoids are selected from the group consisting of:



Please amend claim 5 as follows:

5. ~~The method of~~ any of claims 1, 6, 7, 10, 11, 14, 15, and ~~18~~ wherein said prostanoids are selected from the group consisting of:



Please amend claim 6 as follows:

6. ~~(Amended) A method to assess oxidative stress~~ *in vivo* comprising:

(a) obtaining a [fresh] sample of urine;

(b) measuring the amount of noncyclooxygenase derived prostanoids present in the sample;

(c) comparing said measured amount of prostanoids with a control; and

(d) assessing oxidative stress *in vivo* based on the comparison in step c.

Please amend claim 7 as follows:

7. (Amended) A method to assess oxidative stress *in vivo* comprising:

(a) obtaining a fresh sample of tissue;

(b) measuring the amount of noncyclooxygenase derived prostanoids present in the phospholipids in the sample <u>within about two hours of obtaining the sample, which is the amount of time it takes for the *ex vivo* development of prostanoids in the sample</u>;

(c) comparing said measured amount of [said] prostanoids with a control; and

(d) assessing oxidative stress *in vivo* based on the comparison in step c.

Please cancel claim 8.

Please add claim 10 as follows:

10. ~~A method to assess oxidative stress *in vivo* comprising:~~

(a) obtaining a sample of urine;

(b) measuring the amount of metabolites of noncyclooxygenase derived prostanoids present in the sample;

(c) comparing said measured amount of metabolites of noncyclooxygenase derived prostanoids with a control; and

(d) assessing oxidative stress *in vivo* based on the comparison in step c.

Please add claim 11 as follows:

11. A method to assess oxidative stress *in vivo* comprising:

(a) obtaining a stored sample of lipid containing biological fluid;

(b) measuring the amount of noncyclooxygenase derived prostanoids in the sample;

(c) comparing said measured amount of prostanoids with a control; and

(d) assessing oxidative stress *in vivo* based on the comparison in step c.

Please add claim 12 as follows:

12. The method of claim ~~11~~ 2 wherein the stored sample is maintained at -70°C.

Please add claim 13 as follows:

~~13. The method of claim 11 wherein antioxidants are added to the~~ stored sample ~~prior~~ to its maintenance at -20°C.

Please add claim 14 as follows:

14. A method to assess oxidative stress *in vivo* comprising:

(a) obtaining a fresh sample of lipid containing biological fluid;

(b) subjecting phospholipids in the fresh sample of lipid containing biological fluid to reverse phase solid chromatography extraction using a C-18 cartridge;

(c) eluting prostanoids retained on the C-18 cartridge with a 1:1 mixture of ethyl acetate:heptane;

(d) maintaining the eluate of the phospholipids from the fresh sample of lipid containing biological fluid at -20°C;

(e) measuring the amount of noncyclooxygenase derived prostanoids present in the eluate of the phospholipids from the fresh sample of lipid containing biological fluid maintained at -20°C;

(f) comparing said measured amount of prostanoids with a control; and

(g) assessing oxidative stress *in vivo* based on the comparison in step c.

Please add claim 15 as follows:

15. A method to assess oxidative stress *in vivo* comprising:

(a) obtaining a stored sample of tissue;

(b) measuring the amount of noncyclooxygenase derived prostanoids present in the phospholipids in the sample;

(c) comparing said measured amount of prostanoids with a control; and

(d) assessing oxidative stress *in vivo* based on the comparison in step c.

Please add claim 16 as follows:

16. The method of claim 15 wherein the stored sample is maintained at -70°C.

Please add claim 17 as follows:

17. The method of claim 15 ~~wherein~~ antioxidants are added to the stored sample prior ~~to its~~ maintenance at -20°C.

Please add claim 18 as follows:

18. ~~A method to assess oxidative stress~~ *in vivo* comprising:

(a) obtaining a fresh sample of tissue;

(b) subjecting phospholipids in the fresh sample of tissue to reverse phase solid chromatography extraction using a C-18 cartridge;

(c) eluting prostanoids retained on the C-18 cartridge with a 1:1 mixture of ethyl acetate:heptane;

(d) maintaining the eluate of the phospholipids from the fresh sample of tissue at ~~-20°C~~;

(e) measuring the amount of noncyclooxygenase derived prostanoids present in the eluate of the phospholipids from the fresh sample of tissue maintained at -20°C;

(f) comparing said measured amount of prostanoids with a control; and

(g) assessing oxidative stress *in vivo* based on the comparison ~~in step e~~.

Please add claim 19 as follows:

19. The method of any of claims 1, ~~6, 7,~~ 10, 11, 14, 15, or 18 wherein measurement is by ~~mass~~ spectroscopy.

Please add claim 20 as follows:

20. The method of any of claims 1, 6, 7, 10, 11, 14, 15, or 18 wherein measurement is by immunoassay.

REMARKS

The above changes and additions made to the claims address the Examiner's objections, are corrections, or clarify information contained in the Specification. No new matter has been added.

The Examiner has acknowledged the preliminary amendment and declaration filed on December 5, 1994.

Claims 1 through 9 are now pending in the application. The claimed invention is directed to a method of determining oxidative stress *in vivo* by the quantification of prostaglandin F_2-like oxidative products and prostanoids, in general, that are produced by a noncyclooxygenase, free radical-catalyzed, chemical mechanism of oxidation in either a biological fluid or tissue as well as to specific prostaglandin F_2-like metabolite compounds.

The Examiner has rejected claims 1 through 9 on the basis of 35 USC §103 as being obvious in view of Morrow et al. [Analytical Biochemistry 184:1-10 (1990)] and, therefore, unpatentable. Morrow et al. disclose the discovery that several prostaglandin F_2-like (PGF_2) compounds that are found in the plasma from normal